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Hepatic lipase mutation may reduce vascular disease prevalence in hemodialysis patients with high CETP levels

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Hepatic lipase mutation may reduce vascular disease prevalence in hemodialysis patients with high CETP levels.

Background. Uremic dyslipidemia characterized by reduced high-density lipoprotein (HDL) cholesterol levels is one of the major contributors to the high incidence of cardiovascular disease in hemodialysis patients. Hepatic lipase (HL), together with cholesteryl ester transfer protein (CETP), may not only promote reverse cholesterol transport but also enhance production of small, dense, more atherogenic low-density lipoprotein (LDL). A common C-514T mutation of the promoter region of the HL gene reportedly increases HDL cholesterol levels. However, whether the HL mutation is antiatherogenic or proatherogenic has remained unknown in uremic patients and the general population.

Methods. We investigated the influence of the mutation and its interaction with CETP on HDL cholesterol levels and the apparent atherosclerotic complications in 183 hemodialysis patients aged over 30 years who had received no antilipemic drugs.

Results. In patients with CETP levels ≥2.2 μg/mL [high CETP (HCT) group, $N = 97$], subjects with the TT genotype had a significantly higher level of HDL cholesterol than those without TT genotype (56.8 ± 15.9 mg/dL vs. 45.7 ± 13.4 mg/dL, $P < 0.001$), but not in patients with CETP levels <2.2 μg/mL [low CETP (LCT) group]. Multiple linear regression analysis showed that the TT genotype was a major independent positive determinant for HDL cholesterol levels in the HCT not LCT group. Among the HCT group patients, subjects with the TT genotype ($N = 25$) had a tendency toward lower prevalence of vascular disease than those without TT genotype ($N = 72$) (4.0% vs. 22.2%, $P < 0.07$). In this subgroup, TT genotype had an independent odds ratio of 0.041 (95% CI 0.002 to 0.75, $P < 0.05$) after adjusting for other risk factors.

Conclusion. The TT genotype of HL mutation may serve as a protective factor against vascular disease by increasing HDL cholesterol levels in hemodialysis patients with higher CETP levels.

Key words: dialysis, CETP, HDL cholesterol, atherosclerosis, gene-environment interaction, uremic dyslipidemia.

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The high incidence of atherosclerotic complications is frequently found in patients undergoing maintenance dialysis [1]. Uremic dyslipidemia has been considered a major cause of susceptibility to atherosclerosis in this setting [2, 3], although other factors such as increased oxidant stress, hyperhomocysteinemia, and disorders of calcium and phosphorus metabolism have been suggested [4, 5]. The impaired lipid metabolism is characterized mainly by decreased serum levels of high-density lipoprotein (HDL) cholesterol and decreased reverse cholesterol transport, which result from reduced activity of lipoprotein lipases and lecithin-cholesterol acyltransferase [6].

Hepatic lipase (HL), a 65 kD glycoprotein synthesized and distributed in the liver, catalyses the hydrolysis of triglyceride in native lipoproteins [7]. HL can promote reverse cholesterol transport by converting larger HDL particles back to smaller ones with greater antiatherogenic effects [7, 8], while it not only may decrease HDL cholesterol levels but also may produce low-density lipoprotein (LDL) particles with smaller and denser nature that are more atherogenic [7, 9, 10]. At present, these multiple roles of HL in lipoprotein metabolism make it difficult to determine whether HL action is antiatherogenic or proatherogenic [7]. Indeed, clinical findings on HL-deficient patients were less clear on the impact of HL on atherosclerosis development [7]. Recently, a C- to-T mutation at position -514 (C-514T mutation) of the HL promoter has been identified and shown to be associated with lowered HL activity and elevated HDL cholesterol levels [9, 11]. In several studies, this HL-deficient mutation has not been consistently linked with a risk of coronary heart disease [9, 12, 13].

Cholesteryl ester transfer protein (CETP) can transfer cholesteryl ester (CE) from large CE-rich HDL to very LDL in exchange for triglyceride [8, 14]. This process favors hepatic uptake of CE in HDL and formation of pre-β HDL particles with a high likelihood for cellular

cholesterol efflux, promoting reverse cholesterol transport [8, 14, 15]. However, an elevation in serum CETP leads to reduced HDL cholesterol levels and increased LDL cholesterol levels [16]. As for the general population, several studies have reported that CETP was proatherogenic [17, 18], while other studies have reported that the protein was antiatherogenic [19, 20]. Therefore, the role of CETP in atherosclerosis is not clearly defined. In a large number of hemodialysis patients, we recently reported that high CETP and high HDL cholesterol status serves as a protective factor against vascular disease [21], while low CETP and low HDL cholesterol status is a risk factor for the disease [22]. These findings suggest that the antiatherogenic effects of CETP may be accentuated in dialysis patients.

HL together with CETP may further promote reverse cholesterol transport via enhancing hepatic uptake of CE in HDL and formation of small HDL particles [23–25], although the cooperation also may produce small dense, more atherogenic LDL particles [10, 16, 26] and may further decrease HDL cholesterol levels [7, 16]. Considering that uremic patients frequently have a decrease in both HDL cholesterol levels and reverse cholesterol transport activity [6] and an increase in atherogenic small LDL particles [27], it appears very important to clarify the impacts of HL and its interaction with CETP on atherosclerosis in patients with end-stage renal disease (ESRD). However, there have been no studies investigating whether the HL-deficient C-514T mutation is anti- or proatherogenic in patients receiving dialysis treatment. Furthermore, no information has been collected about the effect of the HL mutation on HDL cholesterol levels in this setting. The present study examined, for the first time, the HL C-514T mutation and its interaction with CETP in relation to atherosclerotic complications in uremic patients. We also identified the homozygote for the HL mutation as a major determinant of HDL cholesterol levels, especially in the hemodialysis patients with high CETP status.

METHODS

Subjects

Between September 1998 and March 1999, we investigated a total of 183 hemodialysis patients aged 30 to 84 years at the dialysis centers in the Fukui and Niigata prefectures. Hemodialysis had been initiated because of ESRD due to chronic glomerulonephritis ($N = 100$), diabetic nephropathy ($N = 33$), polycystic kidney disease ($N = 8$), nephrosclerosis ($N = 12$), chronic pyelonephritis ($N = 13$), toxemia of pregnancy ($N = 1$), a urogenital malformation ($N = 1$), miscellaneous nephropathies ($N = 4$), and shrunken kidney of unknown etiology ($N = 11$). Patients were undergoing three times a week 3- to 5-hour hemodialysis sessions, generally using high-flux membranes and standard heparin doses. The mean Kt/V urea was 1.31 ± 0.25. The mean length of time on hemodialysis was 7.0 ± 7.0 years. Informed consent for study participation was obtained from each subject. Smoking was defined on the basis of current cigarette smoking or previous habitual smoking. Hypertension was defined as a systolic blood pressure >140 mm Hg or a diastolic pressure >90 mm Hg, or the use of antihypertensive drugs. Dietary instructions included daily intake of 30 to 35 kcal/kg and 1.2 g protein/kg and the general suggestion of intake of 55% to 60% carbohydrate, 25% fat, and 15% to 20% protein.

Biochemical assays

Venous blood samples (serum and whole blood) were collected immediately prior to dialysis 2 hours after breakfast or 2 to 3 hours after lunch from all dialysis patients in September 1998. Serum levels of total cholesterol (TC) and triglyceride (TG) were measured by a standard enzymatic method. HDL cholesterol levels were measured in the supernatant after precipitation of other lipoprotein fractions with phosphotungstic acid [28]. Serum albumin levels were determined by the bromocresol green dye-binding assay. Serum CETP concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies (BML, Saitama, Japan). The intra- and interassay coefficients of variation were 2.6% and 5.2%, respectively. We measured postprandial serum levels of lipids and proteins for the following reasons: (*1*) it was very difficult to collect fasting blood samples from all patients because, in our dialysis centers, about half of the patients receive hemodialysis treatment during the afternoon; (*2*) it was reported in healthy subjects that total cholesterol levels were unchanged and HDL cholesterol levels did not change much (less than 10%) after a meal [29]; and (*3*) no significant difference between serum CETP levels during a fast and 2 hours after a meal in healthy subjects ($N = 12$) was observed in our laboratory (2.44 ± 0.16 μg/mL vs. 2.40 ± 0.18 μg/mL, NS).

Assessment of vascular disease

Coronary artery disease. Coronary artery disease was established from a review of medical records. A previous myocardial infarction was defined by a physician's diagnosis based on chest pain, confirmatory electrocardiographic changes, and enzyme determinations or findings from coronary angiography. Angina pectoris was diagnosed when a stress test was positive and/or coronary artery stenosis (>75% of luminal diameter) was demonstrated by angiography. Patients with a history of myocardial infarction or angina pectoris were classified as having coronary artery disease.

Peripheral vascular disease. Peripheral vascular disease was diagnosed by diminished pulses on the patient

history and clinical examinations. Symptoms of intermittent claudication were recorded by a physician. The presence of clinical signs of peripheral vascular disease were determined by a physical examination, including palpation of pulses and auscultation of bruits at the arteries of lower extremities. In the patients with clinical signs, the ankle and arm systolic blood pressures were measured. An ankle/arm index <0.95 indicated the presence of peripheral vascular disease. In the patients with both an index ≥0.95 and clinical signs, including intermittent claudication, angiography was performed. When peripheral artery stenosis (>75% of luminal diameter) was found, the patients were diagnosed as having peripheral vascular disease.

Cerebral vascular disease. Cerebral vascular disease was defined as a stroke or transient ischemic attack [i.e., functional deficit for at least 24 hours and positive findings on computed tomography (presence of hypodensity area) or magnetic resonance imaging (simultaneous presence of hypointensity area on T1-weighted image and hyperintensity area on T2-weighted image), or an acute functional deficit lasting for 24 hours or less], respectively. Patients with cerebral or subarachinoid hemorrhage were excluded.

A patient was considered as having a vascular disease when at least one of these three defined vascular diseases was found.

Determination of the C-514T mutation in the HL gene

Genomic DNA was extracted from peripheral blood leukocytes using a commercial apparatus (MFX-2000) (Toyobo Tsuruga, Fukui, Japan) and a commercially available kit (MagExtractor–Genome) (Toyobo Tsuruga). Identification of the C-514T mutation in the HL gene was performed by polymerase chain reaction (PCR) using a pair of primers as described by Guerra et al [11]. Ten microliters of the 300 bp PCR product was subjected to Nla III digestion (10 U in a 15 μL digest) at 37°C for 3 hours. The digests were electrophoresed on 1.2% agarose gels.

Statistical analysis

Continuous variables are expressed as means ± SD. Differences in continuous variables between patients with and without vascular disease and among three genotype groups were assessed by a nonparametric test (Mann-Whitney U test) and analysis of variance (ANOVA) with multiple comparisons, respectively. Chi-square analysis was used to assess differences in noncontinuous variables between patients with and without vascular disease or among patients with differing genotypes. Multiple linear regression analysis was performed to determine the independent predictors of serum HDL cholesterol levels. Multivariate logistic regression analysis was used to assess the independent contribution of clinical variables including CETP levels and the C-514T mutation in the HL gene to apparent vascular disease in dialysis patients. A level of $P < 0.05$ (two-tailed tests) was considered statistically significant. All statistical analyses were performed using the SPSS statistical software package (SPSS, Inc., Chicago, IL, USA).

Table 1. Characteristics of hemodialysis patients

	All patients (N = 183)
Age *years*	58.9 ± 12.5
Gender *male/female*	107/76
Hemodialysis duration *years*	7.0 ± 7.0
Serum cholesterol *mg/dL*	156 ± 33
Triglyceride *mg/dL*	97 ± 50
HDL-cholesterol *mg/dL*	49 ± 18
Serum albumin *g/dL*	3.7 ± 0.4
CETP *μg/mL*	2.2 ± 0.6
Hypertension (%)	163 (89.1)
Diabetes mellitus (%)	33 (18.0)
Smoking history (%)	79 (43.2)
Vascular disease (%)	38 (20.8)

CETP is cholesteryl ester transfer protein; HLD is high-density lipoprotein.

RESULTS

Table 1 shows clinical and laboratory characteristics of hemodialysis patients. The means of HDL cholesterol and CETP levels were 49 ± 18 mg/dL and 2.2 ± 0.6 μg/mL, respectively. Among a total of 183 hemodialysis patients, 50 subjects were homozygous for the HL C/T mutation (TT genotype), 79 subjects were heterozygous for the mutation (CT genotype), and 54 subjects were the wild-type (CC genotype) as shown in Table 2. The frequency of C and T alleles were 51.1% and 48.9%, respectively. The distribution of HL genotypes agreed with the Hardy-Weinberg equilibrium. No significant differences among the three genotypes were found in any clinical or laboratory characteristics (Table 2). When patients with the CT genotype and those with the CC genotype were analyzed as a single group (those without TT genotype), patients with the TT genotype showed a significantly higher serum level of HDL cholesterol than those without TT genotype (52 ± 18 mg/dL vs. 47 ± 18 mg/dL, $P < 0.05$) (Table 2).

Considering that CETP may influence HDL cholesterol levels, all the patients were divided into four groups according to quartile of CETP concentrations, and HDL cholesterol levels were compared among the CETP subgroups. HDL cholesterol levels were higher in patients with the TT genotype than in those with the CT or the CC genotype for the upper two quartiles: for the highest quartile of CETP (N = 47) (CETP ≥ 2.6 μg/mL, 63 ± 14 mg/dL vs. 51 ± 15 mg/dL vs. 45 ± 14 mg/dL, $P < 0.02$) and for the second (N = 50) (CETP 2.6 μg/mL > CETP ≥ 2.2 μg/mL, 52 ± 16 mg/dL vs. 45 ± 13 mg/dL vs.

Table 2. Characteristics in relation to the hepatic lipase (HL) genotypes

		Non-TT genotypes (N = 133)	
	TT genotype (N = 50)	CT genotype (N = 79)	CC genotype (N = 54)
Age *years*	59.3 ± 13.5	59.5 ± 12.3	57.6 ± 12.0
Gender *male/female*	28/22	47/32	32/22
Hemodialysis duration *years*	5.5 ± 6.5[a]	6.9 ± 7.4	8.4 ± 6.8
Serum albumin *g/dL*	3.6 ± 0.4	3.7 ± 0.3	3.6 ± 0.5
Serum cholesterol *mg/dL*	159 ± 35	156 ± 35	151 ± 29
Triglyceride *mg/dL*	98 ± 61	99 ± 48	93 ± 42
HDL-C *mg/dL*	52 ± 18[b]	48 ± 19	46 ± 15
CETP *μg/mL*	2.2 ± 0.6	2.2 ± 0.6	2.3 ± 0.6
Hypertension (%)	43 (86.0)	70 (88.6)	50 (92.5)
Diabetes mellitus (%)	13 (26.0)[c]	13 (16.5)	7 (13.0)
Smoking history (%)	22 (44.0)	37 (46.8)	20 (37.0)
Vascular disease (%)	6 (12.0)[c]	17 (21.5)	15 (27.8)

CETP is cholesteryl ester transfer protein; HLD is high-density lipoprotein.
[a] $P < 0.1$ non-TT genotype by Mann-Whitney U test
[b] $P < 0.05$ non-TT genotype by Mann-Whitney U test
[c] $P < 0.1$ non-TT genotype by chi-square analysis

41 ± 10 mg/dL, $P < 0.1$), while there were no significant differences in HDL cholesterol levels among the genotypes for the lower two quartiles: for the third (N = 46) (CETP 2.2 μg/mL > CETP ≥ 1.8 μg/mL, 52 ± 24 mg/dL vs. 45 ± 13 mg/dL vs. 51 ± 23 mg/dL, NS) and for the lowest (N = 40) (CETP < 1.8 μg/mL, 43 ± 12 mg/dL vs. 48 ± 23 mg/dL vs. 48 ± 12 mg/dL, NS). Therefore, we selected the median value (2.2 μg/mL) of CETP concentrations as a dividing line to define the high and low CETP subgroups. In the high CETP (≥2.2 μg/mL) subgroup (N = 97), patients with the TT genotype showed a significantly higher serum level of HDL cholesterol than those with the CT genotype and those with the CC genotype (Fig. 1A) and than those without TT genotype (57 ± 16 mg/dL vs. 46 ± 13 mg/dL, $P < 0.001$), while in the low CETP (< 2.2 μg/mL) subgroup (N = 86), the HDL cholesterol levels were similar in patients with differing HL genotypes (Fig. 1B). Multiple linear regression analysis in all the patients identified female gender and TT genotype as positive determinants and triglyceride as a major negative determinant of HDL cholesterol levels (Table 3). In the high CETP subgroup, the TT genotype was a major independent determinant of HDL cholesterol levels after adjustment for all the other clinical variables, including triglyceride (Table 4), while in the low CETP subgroup, it showed no independent association with HDL cholesterol levels (data not shown).

Next, we examined the effects of the HL TT genotype on the prevalence of apparent vascular disease in hemodialysis patients. Of all the patients (N = 183), a total of 38 subjects (20.8%) had apparent vascular disease. Coronary artery disease, peripheral vascular disease, and cerebral vascular disease were identified in 17, 4, and 24 patients, respectively. As shown in Table 5, patients with vascular disease were older and had a shorter duration of hemodialysis, lower serum levels of HDL cholesterol

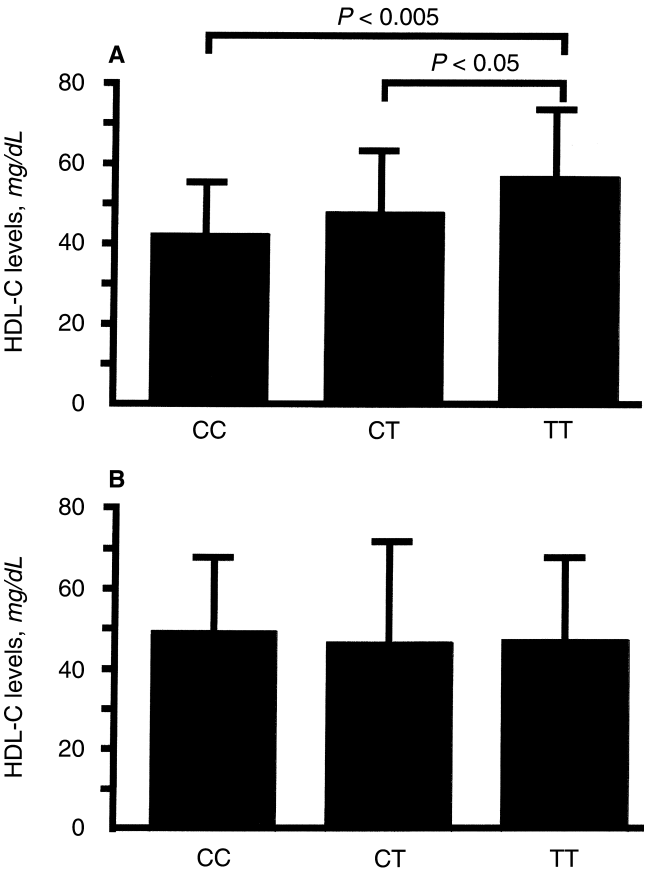

Fig. 1. High-density lipoprotein (HDL) cholesterol levels according to the hepatic lipase (HL) genotypes among patients taking no antilipemic drugs. (*A*) In patients with cholesteryl ester transfer proteins (CETP) levels ≥2.2 μg/mL (N = 97), those with the TT genotype had a significantly higher level of HDL cholesterol than those with the CT and those with the CC genotype (56.8 ± 15.9 mg/dL vs. 48.0 ± 14.1 mg/dL vs. 42.6 ± 12.0 mg/dL, $P < 0.005$). (*B*) In patients with CETP levels <2.2 μg/mL (N = 86), those with differing genotypes did not differ in HDL cholesterol levels.

Table 3. Multiple regression analysis of variables affecting serum high-density lipoprotein (HDL) cholesterol levels in hemodialysis patients[a]

Variables	Standard regression coefficients	*P* value
Age *years*	−0.041	0.3273
Gender (male = 0, female = 1)	0.164	0.0308
Diabetes mellitus (presence = 1, absence = 0)	−0.183	0.0100
Smoking (presence = 1, absence = 0)	−0.029	0.7027
Hemodialysis duration *years*	0.018	0.8033
Triglyceride *mg/dL*	−0.380	<0.001
Serum albumin *g/dL*	0.039	0.5827
CETP *μg/mL*	−0.041	0.5569
HL genotypes (CC, CT = 0, TT = 1)	0.155	0.0219
R^2	0.252	<0.001

CETP is cholesteryl ester transfer protein.
[a] R^2 is mulitple coefficient of determination

Table 4. Multiple regression analysis of variables affecting a serum high-density lipoprotein (HDL) cholesterol levels in hemodialysis patients with cholesteryl ester transfer protein (CETP) levels ≥2.2 μg/mL[a]

Variables	Standard regression coefficients	*P* value
Age *years*	−0.167	0.0770
Gender (male = 0, female = 1)	0.200	0.0456
Diabetes mellitus (presence = 1, absence = 0)	−0.157	0.0763
Smoking (presence = 1, absence = 0)	−0.175	0.0810
Hemodialysis duration *years*	−0.092	0.3177
Triglyceride *mg/dL*	−0.364	<0.001
Serum albumin *g/dL*	0.140	0.1159
HL genotypes (CC, CT = 0, TT = 1)	0.342	<0.001
R^2	0.435	<0.001

[a] R^2 is mulitple coefficient of determination

and albumin, and a higher prevalence of diabetes mellitus than those without vascular disease. Patients with the TT genotype had a tendency toward lower prevalence of vascular disease than those without TT genotype for the entire group (12.0% vs. 24.1%, $P < 0.1$) (Table 2) and for the high CETP subgroup (4.0% vs. 22.2%, $P < 0.07$) (Fig. 2A), while for the low CETP subgroup, prevalences of vascular disease were similar in patients with and without TT genotype (Fig. 2B).

In addition to HDL cholesterol levels, patients with and without TT genotype differed in some other characteristics (Tables 2 and 6). As for the entire group, patients with the TT genotype had a tendency toward shorter duration of hemodialysis and higher prevalence of diabetes mellitus than those without TT genotype (Table 2). As for the high CETP subgroup, patients with the TT genotype had a significantly higher level of total cholesterol than those without TT genotype (Table 6). Therefore, we finally investigated the independent influences of the TT genotype on vascular disease in hemodialysis patients using multiple logistic regression analysis in a model, including age (years), gender, duration of hemodialysis (years), total cholesterol (mg/dL), serum albumin (g/dL), HDL cholesterol (mg/dL), CETP (μg/mL), as well as the presence or absence of hypertension, diabetes mellitus, smoking history, and TT genotype in the HL gene. Multivariate analysis in the entire group identified the TT genotype [odds ratio (OR), 0.19; 95% confidence interval (95% CI), 0.06 to 0.62; $P < 0.01$] as significant independent predictors for vascular disease (Table 7). In the high CETP subgroup, the TT genotype was again selected as a significant predictor [OR, 0.041 (95% CI, 0.002 to 0.75; $P < 0.05$)] after adjustment for other risk factors, while in the low CETP subgroup the TT genotype was not an independent predictor for vascular disease (Table 7).

DISCUSSION

We studied for the first time the HL C-514T mutation in uremic patients on maintenance hemodialysis. The frequency of the mutant allele in the present Japanese patients (T allele, 48.9%) was similar to that recently reported for the general population of Japanese males [30] and our preliminary findings for Japanese healthy subjects (49.1%, other detailed data not shown) and was about threefold higher than those previously reported for American and European general populations [9].

One of the most notable observations from the present study is that the TT genotype for HL C-514T mutation is a major positive determinant for HDL cholesterol levels in hemodialysis patients with high CETP levels (≥2.2 μg/mL) but not in those with low CETP levels (<2.2 μg/mL). Since HL readily hydrolyzes TG in large TG-rich HDL and then increases the HDL clearance rate from the blood stream by enhancing not only hepatic uptake of large HDL particles but also conversion of larger TG-rich HDL ones to smaller HDL ones [7, 9, 24], low HL activity results in increased HDL cholesterol levels [7, 9]. Recently, the HL C-514T mutation was reported to be associated with reduced HL activity and to cause increased HDL cholesterol levels, which was also supported by our study, especially large TG-rich HDL particles [9, 11]. Subjects with the TT genotype have lower HL activity and higher HDL cholesterol levels than those with a non-TT genotype [9, 11]. Since CETP preferably forms the large TG-rich HDL from the large CE-rich one via exchange of CE for TG [14, 16] and also elevates serum lecithin:cholesterol acytransferase (LCAT) activity [31], the production rate of large TG-rich HDL is necessarily higher in high CETP status than in low CETP status. Considering that the large TG-rich HDL is a better substrate for HL [7], in the high CETP subgroup where TG-rich HDL is abundant, patients with a non-TT genotype may have higher HDL clearance than those with the TT genotype, while in the

Table 5. Characterisitcs of hemodialysis patients with and without vascular disease (VD)[a]

	All patients (N = 183)		Patients with CETP levels ≥2.2 μg/mL (N = 97)	
	VD (+) (N = 38)	VD (−) (N = 145)	VD (+) (N = 17)	VD (−) (N = 80)
Age *years*	68.1 ± 10.7[b]	56.5 ± 11.9	69.2 ± 12.6[b]	56.9 ± 11.5
Gender *male/female*	24/14	83/62	9/8	41/39
Hemodialysis duration *years*	3.3 ± 4.3[b]	7.9 ± 7.3	2.6 ± 2.9[b]	9.0 ± 7.7
Serum cholesterol *mg/dL*	157 ± 36	155 ± 33	161 ± 30	158 ± 31
Triglyceride *mg/dL*	110 ± 63	94 ± 46	98 ± 42	93 ± 41
HDL-cholesterol *mg/dL*	42 ± 15[b]	50 ± 19	40 ± 10[c]	50 ± 15
Serum albumin *g/dL*	3.6 ± 0.4[c]	3.7 ± 0.4	3.6 ± 0.3[c]	3.7 ± 0.4
CETP *μg/mL*	2.1 ± 0.5	2.3 ± 0.6	2.5 ± 0.3	2.7 ± 0.5
Hypertension (%)	36 (94.7)	127 (87.6)	16 (94.1)	70 (87.5)
Diabetes mellitus (%)	13 (34.2)[b]	20 (13.8)	6 (35.3)[c]	9 (11.3)
Smoking history (%)	15 (39.5)	64 (44.4)	6 (35.3)	37 (46.2)

HDL is high density lipoprotein; CETP is cholesteryl ester transfer protein.

[a] VD (+) and VD (−) indicate patients with and without vascular disease, respectively. Differences in continuous and categoric variables between VD (+) and VD (−) were assessed by Mann-Whitney U test and by chi-square analysis or Fisher's exact probability test, respectively

[b] $P < 0.01$ vs. VD (−) in each patient group; [c] $P < 0.05$, vs. VD (−) in each patient subgroup

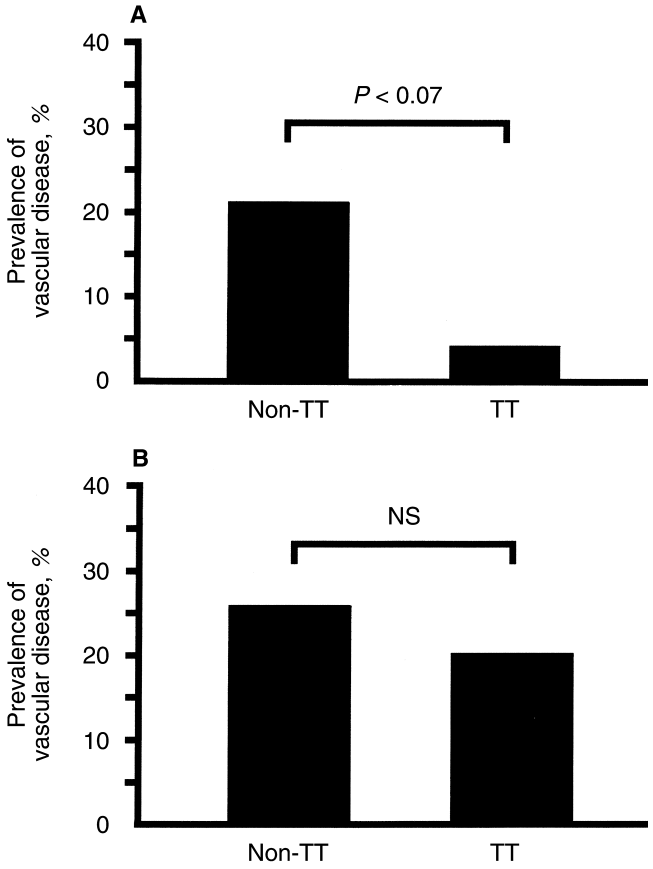

Fig. 2. Vascular disease prevalence according to the hepatic lipase (HL) genotype. The non-TT genotypes include both the CC and CT genotypes. (*A*) In patients with cholesteryl ester transfer proteins (CETP) levels ≥2.2 μg/mL (N = 97), those with TT genotype had a tendency toward lower prevalence of vascular disease than those with a non-TT genotype (4.0% vs. 22.2%, $P < 0.07$). (*B*) In patients with CETP levels <2.2 μg/mL (N = 86), there was a similar prevalence of vascular disease in the TT and non-TT genotypes (20.0% vs. 26.2%, NS).

Table 6. Characteristics of patients with TT or non-TT genotypes in a high cholesteryl ester transfer protein (CETP) subgroup[a]

	TT genotype (N = 25)	Non-TT genotypes (N = 72)
Age *years*	60.2 ± 11.9	58.6 ± 12.8
Gender *male/female*	15/10	35/37
Hemodialysis duration *years*	6.8 ± 7.4	8.2 ± 7.5
Serum albumin *g/dL*	3.6 ± 0.4	3.6 ± 0.4
Serum cholesterol *mg/dL*	169 ± 32[b]	155 ± 29
Triglyceride *mg/dL*	85 ± 29	97 ± 44
HDL-cholesterol *mg/dL*	57 ± 16[c]	46 ± 13
CETP *μg/mL*	2.6 ± 0.4	2.7 ± 0.5
Hypertension (%)	21 (84.0)	65 (90.3)
Diabetes mellitus (%)	4 (16.0)	11 (15.3)
Smoking history (%)	14 (56.0)	29 (40.3)
Vascular disease (%)	1 (4.0)[d]	16 (22.2)

HDL is high-density lipoprotein.

[a] Non-TT genotypes include both CT genotype and CC genotype. A high CETP subgroup includes patients with CETP levels ≥2.2 μg/mL

[b] $P < 0.05$ non-TT genotypes by MannnWhiteny U-test

[c] $P < 0.01$ non-TT genotypes by Mann-Whitney U-test

[d] $P < 0.07$ non-TT genotypes by Fisher's exact probability test

low CETP subgroup where TG-rich HDL is less, the HL-mediated HDL clearance may be similar among subjects with differing HL genotypes. Consequently, the HDL-raising effect of the TT genotype may be more conspicuous in the high CETP subgroup than in the low CETP one. At present, however, the presence of the interaction between the HL mutation and CETP antigen in the general population and the precise mechanism remains to be clarified.

Another notable finding in the present study was that the TT genotype in the HL mutation was an independent protective factor against vascular disease in the high CETP subgroup but not in the low CETP subgroup. It is highly likely that this antiatherogenic effect of the TT genotype arises mainly from its association with the marked increase in HDL cholesterol levels (Fig. 1A). In

Table 7. Multiple logistic regression analysis of the influence of selected variables on apparent vascular disease in hemodialysis patients[a]

Variables	Regression coefficient	SE	Odds ratio (95% CI)	*P* value
All patients[b]				
Age	0.090	0.023	1.09 (1.05–1.14)	<0.001
HL TT genotype	−1.657	0.605	0.19 (0.06–0.62)	<0.01
Diabetes mellitus	1.386	0.566	4.00 (1.32–12.13)	<0.05
HDL cholesterol	−0.032	0.016	0.97 (0.94–0.99)	<0.05
Patients with CETP ≥2.2 μg/mL[c]				
HL TT genotype	−3.190	1.483	0.041 (0.002–0.75)	<0.05
Age	0.065	0.034	1.07 (1.00–1.14)	0.056
Duration of hemodialysis	−0.174	0.092	0.83 (0.69–1.00)	0.059
Patients with CETP <2.2 μg/mL[c]				
Age	0.122	0.037	1.13 (1.05–1.21)	<0.001
Diabetes mellitus	1.463	0.806	4.32 (0.89–21.10)	0.069

HDL is high-density lipoprotein; CETP is cholesterol ester transfer protein.

[a] Variables with *P* values less than 0.1 are listed

[b] Variables are age (years), gender (female = 0, male = 1), duration of hemodialysis (years), serum cholesterol (mg/dL), serum albumin (g/dL), HDL-cholesterol (mg/dL), CETP (μg/mL), and absence (0) or presence (1) of hypertension, diabetes mellitus, history of smoking, and HL TT genotype

[c] Variables are all those used for analysis of all patients except for CETP (μg/mL)

general, increased HDL cholesterol levels have been recognized as a protective factor against atherosclerotic complications in uremic patients [22, 32–34] as well as general populations [35–37]. In our study, considering all the patients, HDL cholesterol (mg/dL) was significantly and inversely associated with vascular disease prevalence after adjustment for all the other clinical variables including the TT genotype (Table 7), while in the high CETP subgroup, the TT genotype but not HDL cholesterol was selected as an independent predictor for vascular disease. These findings may reflect much stronger association of the TT genotype with increased HDL cholesterol levels in high CETP subgroup than in the entire group. Additionally, considering that for all the patients the TT genotype has an inverse relationship with vascular disease prevalence after adjustment for both CETP and HDL cholesterol concentrations, it would seem that the HL mutation contributes partially to vascular disease risk through a certain mechanism other than its HDL cholesterol increasing effect.

We previously reported that CETP serves as a protective factor against vascular disease in hemodialysis patients with normal to high HDL cholesterol levels [21]. As discussed above, HL together with CETP may promote not only hepatic uptake of HDL cholesterol but also conversion of larger HDL particles to smaller ones with greater potential for cellular cholesterol efflux, leading to increased reverse cholesterol transport activity [7, 8, 23, 24]. Thus, it was at first suggested that simultaneous elevation of both CETP and HL would be a more protective factor against vascular disease. However, in contrast to our expectation, we observed in the current study that hemodialysis patients with high CETP levels and the TT genotype leading to the lowest HL activity had the lowest prevalence of vascular disease. This suggests that low HL activity may be a protective factor against vascular disease in high CETP status. HL has been shown to have bidirectional roles on atherosclerosis development [7, 9]. Although HL may promote remnant lipoprotein clearance and reverse cholesterol transport [7, 9], its activity is inversely correlated with HDL cholesterol levels [7, 9]. Several recent studies have reported that HL in cooperation with CETP may enhance formation of small, dense LDL, which has more atherogenic potential [10, 16, 26]. Furthermore, low HL activity resulting from the TT genotype for the HL C/T mutation or from drug therapy with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors is reportedly associated with more buoyant, less atherogenic LDL particles [38, 39] and with regression of coronary heart disease [39]. In uremic patients, increased serum levels of small dense LDL have been found and recently recognized as a risk factor for vascular disease [27, 40, 41]. In the high CETP subgroup, hemodialysis patients with the TT genotype might have had lower levels of small dense LDL as well as actually higher levels of HDL cholesterol than those with a non-TT genotype, possibly leading to a much lower prevalence of vascular disease in the former than in the latter, although patients with the TT genotype had apparently, but not significantly, lower postprandial TG levels than those without TT genotype.

Recently, several studies showed a relationship between the HL C/T mutation and impaired glucose tolerance despite little understanding of the mechanisms [42, 43]. In our study, patients with the TT genotype had a higher prevalence of diabetes mellitus than those without TT genotype with marginal significance for the whole group (Table 2), with significance for the low CETP subgroup (36.0% vs. 14.8%, $P < 0.05$) and with no significance for the high CETP subgroup (Table 6). Although our these findings supported the association of the HL mutation with impaired glucose tolerance [42, 43] and for the first time suggested that the genetic effect may be modified by CETP status, further examinations

are needed to clarify the more susceptibility to diabetes mellitus in individuals with the TT genotype and the interaction between the HL mutation and CETP antigen.

In our study, most serious limitations are the lack of fasting samples. To overcome this shortcoming, we reanalyzed the impact of HL mutation on HDL cholesterol levels and vascular disease prevalence using other two data sets of nonfasting lipid levels (total cholesterol, HDL cholesterol, and TG) measured on previous different days (in October 1998 and in November 1998). Consequently, we were again able to obtain results similar to those shown in Fig. 1 and Tables 2 to 7. Using one set of lipid data, significant differences in HDL cholesterol levels between the HL (TT, CT, and CC) genotypes were found in the highest quartile of CETP (57 ± 9.2 mg/dL vs. 47 ± 11 mg/dL vs. 41 ± 16 mg/dL, $P < 0.01$) and in the second (51 ± 13 mg/dL vs. 44 ± 13 mg/dL vs. 40 ± 18 mg/dL, $P < 0.05$), while the HL genotypes did not differ in HDL cholesterol levels in the third (51 ± 13 mg/dL vs. 42 ± 13 mg/dL vs. 51 ± 18 mg/dL, NS) and in the lowest (43 ± 11.2 mg/dL vs. 45 ± 19 mg/dL vs. 43 ± 11 mg/dL, NS). As for another set of lipid data, significant differences in HDL cholesterol levels between the genotypes were also found in the highest (58 ± 10 mg/dL vs. 44 ± 12 mg/dL vs. 42 ± 14 mg/dL, $P < 0.005$) and in the second (50 ± 11 mg/dL vs. 42 ± 11 mg/dL vs. 39 ± 10 mg/dL, $P < 0.05$), while the genotypes did not differ in HDL cholesterol levels in the third (48 ± 16 mg/dL vs. 45 ± 13 mg/dL vs. 51 ± 20 mg/dL, NS) and in the lowest (42 ± 13 mg/dL vs. 46 ± 16 mg/dL vs. 41 ± 9.2 mg/dL, NS). Furthermore, multiple linear regression analyses using these lipid data proved that the TT genotype was a major positive determinant of HDL cholesterol levels for all the patients and for the patients with high CETP but not for those with low CETP (data not shown). These findings strongly reconfirmed the reported association of the HL mutation with HDL cholesterol levels (Fig. 1 and Tables 2 to 4 and 6). As for the relationship between the HL mutation and vascular disease prevalence, multiple logistic regression analyses using these lipid data revealed a significant inverse relationship between the HL TT genotype and vascular disease prevalence for all the patients and for the patients with high CETP but not for those with low CETP after adjustment of all the other variables including HDL cholesterol and CETP (data not shown), which was consistent with results shown in Table 7.

Several other limitations exist in the current study. Only apparent, but not subclinical, vascular disease at one time point was identified. It would have been preferable to measure fasting lipid levels and to examine HDL or LDL particle composition, including small dense LDL particles. Presumed association of the HL TT genotype with high prevalence of diabetes would have biased the HL genotype distribution due to premature death. Therefore, a large-scale, prospective, longitudinal study with detailed examinations on HDL and LDL particle composition and atherosclerosis status is required to completely define the effect of the HL C-514T mutation on atherosclerotic vascular disease.

CONCLUSION

The TT genotype for the HL C-514T mutation is an independent positive determinant of serum HDL cholesterol levels and may serve as a protective factor against vascular disease independent of traditional risk factors in hemodialysis patients with supramedian CETP levels (≥2.2 μg/mL). These findings suggest the important involvement of hepatic lipase on lipoprotein metabolism and vascular disease in uremic patients with high CETP status.

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